LYMPHATIC RESEARCH AND BIOLOGY
Volume 9, Number 2, 2011

DOI: 10.1089/lrb.2010.0020

Bioimpedance in the Assessment of Unilateral Lymphedema of a Limb: The Optimal Frequency

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Abstract

Background: Bioimpedance techniques provide a reliable method of assessing unilateral lymphedema in a clinical setting. Bioimpedance devices are traditionally used to assess body composition at a current frequency of 50 kHz. However, these devices are not transferable to the assessment of lymphedema, as the sensitivity of measuring the impedance of extracellular fluid is frequency dependent. It has previously been shown that the best frequency to detect extracellular fluid is 0 kHz (or DC). However, measurement at this frequency is not possible in practice due to the high skin impedance at DC, and an estimate is usually determined from low frequency measurements. This study investigated the efficacy of various low frequency ranges for the detection of lymphedema.

Methods and Results: Limb impedance was measured at 256 frequencies between 3 kHz and 1000 kHz for a sample control population, arm lymphedema population, and leg lymphedema population. Limb impedance was measured using the ImpediMed SFB7 and ImpediMed L-Dex® U400 with equipotential electrode placement on the wrists and ankles. The contralateral limb impedance ratio for arms and legs was used to calculate a lymphedema index (L-Dex) at each measurement frequency. The standard deviation of the limb impedance ratio in a healthy control population has been shown to increase with frequency for both the arm and leg. Box and whisker plots of the spread of the control and lymphedema populations show that there exists good differentiation between the arm and leg L-Dex measured for lymphedema subjects and the arm and leg L-Dex measured for control subjects up to a frequency of about 30 kHz.

Conclusions: It can be concluded that impedance measurements above a frequency of 30 kHz decrease sensitivity to extracellular fluid and are not reliable for early detection of lymphedema.

Introduction

Bioimpedance devices are rapidly gaining momentum as an adjunct in the early detection of unilateral limb lymphedema. Lymphedema is characterized by an increase in extracellular fluid. Traditional methods of assessment include circumferential measurements and volume by calculation, perometry, or Archimedes principle. However, each method measures only the total fluid volume of the limb and is not sensitive to changes in extracellular fluid. Bioimpedance techniques (both single frequency and bioimpedance spectroscopy, BIS) can measure changes in extracellular fluid and have been used to assess unilateral lymphedema.[1,2] In this case, the variation from "normal" of the ratio of the affected or at risk limb impedance to the healthy contralateral limb impedance is used to assess the degree of lymphedema present.

Bioimpedance devices measure the electrical impedance of biological tissue in response to an applied alternating current. The electric current will pass through different tissues dependent upon the impedance to flow. Electric currents applied to the body are primarily distributed in fluids and blood, due to the low resistivity of these tissues. In the presence of lymphedema, the applied current will travel predominantly through the accumulation of lymphatic fluids. This noninvasive and cost-effective technology can be used as a reliable tool to assess the presence of lymphedema in patients with either upper or lower limb affliction at an early stage.[3]

The electrical properties of tissue can be described by electrically similar components. When current is applied to the tissue, the extra- and intracellular fluids behave as resistive components, while the cell membranes behave as reactive components. At 0 kHz (direct current or DC) the cell membrane

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The research studies have been funded by in kind support from ImpediMed Ltd, Brisbane, Australia, with clinical measurements of test subjects obtained independently by Dr. R. Box.

acts as an insulator and all current flows through the extracellular fluid. The impedance measured at DC (R_0) is that of extracellular fluid only. At higher current frequencies (alternating current or AC), the cell membrane becomes a conductor and the current will flow in both the extracellular and intracellular fluid in a ratio that is dependent of the applied current frequency. The impedance measured at a single frequency is therefore a combination of the contribution from extra- and intracellular fluid. As the frequency increases, there will become a practical frequency at which the measured impedance is no longer sensitive enough to track extracellular fluid alone.

Due to practical limitations, R_0 cannot be measured directly. However, knowledge of the frequency response of biological material allows finite frequency data to be extrapolated to derive this value. Bioimpedance spectroscopy (BIS) devices that use up to 256 frequency measurements to extrapolate to the DC value have been shown to be more sensitive to extracellular fluid changes.[4] It has been shown that R_0 is most sensitive to changes in extracellular fluid[5] and is therefore the best index of lymphedema.

It has been suggested that single frequency impedance limb ratios and R_0 limb ratios are essentially interchangeable in assessing lymphedema as long as the current frequency remains low.[6] However, the same study also reports an increase in the mean difference between the R_0 and single frequency methods and a widening of the 95% confidence interval of the bias as the measurement frequency of the device increased. The study does not address a specific cut-off frequency for accurate assessment of lymphedema. The confidence interval increases from −1.2% to 2.8% at 10 kHz to −4% to 7.1% at 50 kHz, approximately 250% increase in interval width. This suggests that lymphedema assessments using a current frequency of 50 kHz do not have adequate sensitivity. The study also highlights the need for appropriate detection ratios for leg lymphedema. In another study, a frequency range of 5–10 kHz has been identified as the optimum for single frequency measurement of lower leg swelling due to blood pooling.[7]

Many single frequency bioimpedance devices (predominantly 50 kHz) are available on the market. These devices are traditionally used to determine body composition parameters, including total body water and extracellular water of the whole body. However, at 50 kHz, the current passes through both intra- and extracellular fluid and so the determination of the fluid levels are not dependant on extracellular fluid alone.

Body composition devices are also designed in most cases to measure hand to foot impedance to determine whole body composition using prediction equations based on population studies. The hand to foot measurement consists of the addition of the arm, trunk, and leg impedance. Whole body water content (measured using specific total body water apparatus measured at 50 kHz) has been shown to be insensitive to decreases in circumferential and volumetric measures of an arm during the treatment of lymphedema.[8] Therefore, changes in extracellular fluid volume in a single limb would be better identified by a single limb measurement. Bioimpedance has been shown to reliably measure specific body segments (arms and legs) when the measurement electrodes are positioned on the contralateral limb, thus using the concept of electrical equipotentials. This approach enables accurate and reproducible results.[9]

Therefore the aim of this study is to demonstrate the frequency dependence of the precision of unilateral lymphedema assessment using bioimpedance techniques and to investigate the efficacy of various frequency ranges for the accurate discrimination between lymphedema and control subjects. This will lead to a more informed and reliable choice in the selection of current frequency for the assessment of lymphedema.

Materials and Methods

In order to determine the dependence of the assessment of unilateral lymphedema on the current frequency used to measure the bioelectrical impedance, the impedance of each limb was measured at 256 frequencies. This was completed for a control population and populations with clinically evident unilateral lymphedema in either the arm or the leg, classified according to a criterion referenced scale modified from that described by Miller et al.[10] The data used in this study was collected as part of three research studies conducted with the Queensland University of Technology (QUT) and the University of Queensland (UQ). Ethics approval was obtained from the respective authoritative bodies and informed consent was obtained from subjects prior to participation. Each data subset was used for analysis of one of the three sample population groups. These were the control population, unilateral arm lymphedema population, and unilateral leg lymphedema population.

The limb impedance of each participant was measured using either the ImpediMed L-Dex U400 or the ImpediMed SFB7, according to standard measurement procedures. The L-Dex U400 instrumentation implements the same BIS platform utilized in the SFB7 to measure the raw bioimpedance data, thus generating equivalent raw impedance values. The differences between the two devices are aesthetic where the software and user interface of the L-Dex U400 have been specifically designed for use in assessing lymphedema in a clinical environment. Both devices measure the impedance at 256 frequencies from 3 kHz to 1000 kHz and perform regression analysis to determine R_0 and R_∞(the theoretical resistance at infinite frequency).

Limb impedance was measured using an equipotential electrode placement on the wrists and ankles.[9] The limb impedance ratio was calculated for all 256 current frequencies. For the control population, the nondominant to dominant limb ratio was calculated. For the test populations, the unaffected to affected limb ratio was calculated. The limb impedance ratio was then used to determine a lymphedema index (L-Dex), used to assess the presence of lymphedema. In the case of SFB7 measurements, the raw impedance was converted to an L-Dex score using proprietary software supplied by the manufacturer.

The L-Dex is a quantitative indicator that represents the comparison of the measured limb impedance ratio to a 'normal' range of limb impedance ratios established in a healthy population matched for limb dominance and gender. This is unlike body composition algorithms that use prediction equations to determine the amount of extracellular water content from impedance measurements. The simple linearization of the normal distribution means that measured impedance ratios equal to the mean ratio of the equivalent healthy population will have an L-Dex of 0 and those greater

than three standard deviations from the equivalent healthy population range will have an L-Dex greater than 10. The use of the L-Dex for the assessment of lymphedema has been previously reported.[11,12] These studies identify the need for a simple diagnostic scale to be used in the assessment of lymphedema. The use of L-Dex achieves this and allows the results to be directly compared across gender and dominance of the affected limb on one simple scale. This avoids the need for separate analysis to be performed for different population characteristics (eg, female dominant arm affected) that is required when working with raw impedance ratios.

Arm lymphedema population

The sample unilateral arm lymphedema population data was collected at the Queensland Lymphedema and Breast Oncology Physiotherapy Clinic (UQ ethics approval No. 2007001013). Data was collected from all eligible and consenting patients previously diagnosed with arm lymphedema who attended the clinic during the study duration. Data collection for patients with mild to severe unilateral arm lymphedema secondary to breast cancer surgery was conducted using the ImpediMed L-Dex U400 and current clinical practice to create a dataset indicative of a sample arm lymphedema test population. The demographics of the arm lymphedema population are shown in Table 1. The impedance of both arms was measured at 256 current injection frequencies and the unaffected/affected arm impedance ratio was calculated and the L-Dex recorded. The clinical characteristics of the lymphedema were graded according to the modified scale from that of Miller et al.[10] and the ImpediMed XCA. Of the 15 subjects recruited into this population group, only data from 12 subjects were analyzed, based on the criteria that at least two of the three clinical assessment methods must confirm the presence of lymphedema at the time of assessment.

Leg lymphedema population

The sample unilateral leg lymphedema population was collected at the Queensland Lymphedema and Breast Oncology Physiotherapy Clinic (UQ ethics approval No. 2008001030). Data was collected from all eligible and consenting patients previously diagnosed with leg lymphedema who attended the clinic during the study duration. Data collection for patients with mild to severe unilateral leg lymphedema was conducted using the ImpediMed L-Dex U400 and current clinical practice to create a dataset indicative of a sample leg lymphedema test population. The demographics of the leg lymphedema population are shown in Table 1. The impedance of both legs was measured at 256 current frequencies and the unaffected/affected leg impedance ratio was calculated and the L-Dex recorded. The clinical characteristics of the lymphedema were graded according to the scale modified from that of Miller et al.[10] and circumferential volume differences using a tape measure. Of the 16 subjects recruited into this population group, only the data from 12 subjects was analysed, based on the criteria that at least two of the three clinical assessment methods must confirm the presence of lymphedema at the time of data collection.

Control population

The control population data was collected at the Queensland University of Technology (QUT ethics approval No. 0700000853). A survey of the healthy population was conducted using the ImpediMed SFB7 for the limb impedance ratios at 256 current frequencies between 3 kHz and 1000 kHz. Sixty-five self-diagnosed healthy females who met eligibility criteria were recruited from the staff and student population. The demographics of the control population are shown in Table 1. The impedance of both arms and legs was measured and the nondominant/dominant arm and leg impedance ratio and L-Dex was calculated using the proprietary software supplied by the manufacturer

Results and Discussion

The mean subject age was significantly higher in the test populations than the control population ($p < 0.0001$). The difference of the mean height between each population and the mean weight between each population was not significant (arms: $p = 0.1874$, legs: $p = 0.9453$, and arms: $p = 0.4472$, legs: $p = 0.6163$ for height and weight, respectively).

The arm and leg impedance ratios of the control and test populations were calculated for each frequency between 3 kHz and 1000 kHz. The mean and standard deviation of the impedance ratio was also calculated at each frequency, as well as R_0 and R_∞. The variation of the standard deviation for the arm and leg impedance ratios of the control population as a function of current measurement frequency is shown in Figure 1. The standard deviation of the arm impedance ratio at 0 kHz is 0.031. This is comparable to other published examples that report the standard deviation of female arm ratios to be 0.034.[13] It is hypothesized that the lower standard deviation in the present study is due to advancements in instrumentation technology that produces more reliable and repeatable measurements. The 25^{th}–75^{th} interquartile range of the nondominant to dominant limb impedance ratio of a healthy control subject has also been reported as 0.994–1.057

Table 1. Population Demographics for Control, Arm Lymphedema, and Leg Lymphedema Subjects

Parameter ($\mu \pm \sigma$)	*Control Group*	*Arm Lymphoedema Group*	*Leg Lymphoedema Group*
No. of Subjects	65	12	12
Age (years)	40.8 ± 11.6	61.2 ± 9.2	60.5 ± 6.5
Height(cm)	165.1 ± 6.3	162.7 ± 3.1	165.0 ± 7.1
Weight (kg)	70.5 ± 14.6	74.0 ± 13.8	72.7 ± 11.5
Type of lymphedema	N/A	12 Secondary	9 Secondary/3 Primary
Modified Millers Grading	4 ± 0	10.3 ± 2.2	9 ± 1.7
L-Dex Score	0 ± 3.3	44.0 ± 37.8	24.8 ± 14.4

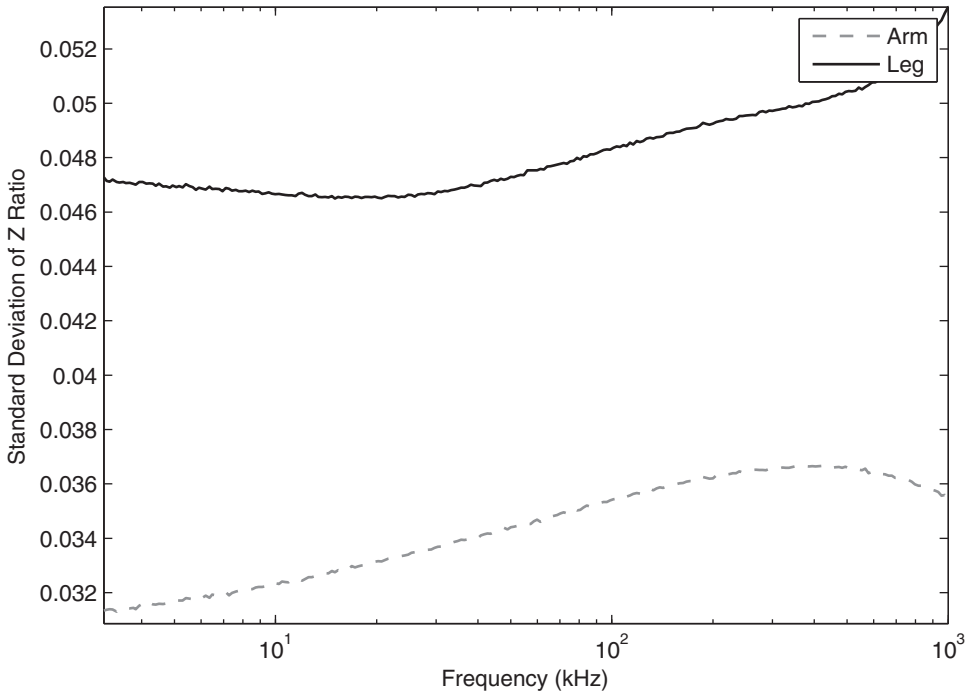

FIG. 1. Standard deviation of impedance ratio for female arms (- -) and legs (-) as a function of current injection frequency.

(0.063).[2] This suggests a 50% distribution (in comparison to 68% for one standard deviation) of ± 0.0315, which is comparable to the standard deviation found by this current study. There has been no standard deviation previously published for healthy leg impedance ratios.

The standard deviation for the leg ratio is consistently higher than for the arm ratio. This is due to better accuracy in measuring an arm. In the case of the leg, the geometry of the body means that often the legs cannot be separated enough to provide adequate insulation between the thighs. As a result, impedance measurements of the leg may contain more noise than the arm due to this added interference.

Figure 1 clearly shows that the standard deviation of the limb impedance ratio of a normal healthy population increases as the current frequency increases for both the arm and the leg. This is more evident in the standard deviation of the arm ratios which increases from 3 kHz. The standard deviation of the leg ratios begins to increase more rapidly at 20 kHz. These results indicate that the natural spread of the measured impedance ratio within a normal population increases with the measurement frequency.

The variance of the limb impedance ratio is expected to be smallest at 0 kHz; however, this is not the case for the legs. In the legs, the variance decreases from 0.047at 0 kHz to 0.046 at 20 kHz. Again, this may be due to inherent noise in a leg measurement induced by the geometry of the legs. Additionally, the nonbalanced nature of the measuring devices results in large differences for an arm or a leg between the fit of the raw data to a Cole model over the frequency range. This is an interesting result and should be investigated further.

The assessment of unilateral lymphedema is performed by comparing the measured impedance ratios for patients with lymphedema to the average ratio and the standard spread of the ratio collected for a normal healthy population. The L-Dex parameter allows impedance ratios to be compared across genders and limb dominance on the same scale and is used in the remainder of the analysis. The agreement between bioimpedance indices (L-Dex) and interlimb volume differences, as determined by perometry, for assessment of unilateral arm lymphedema has been reported.[12] Arm impedance and volume was measured in 45 women with lymphedema and 21 women as part of a control group without lymphedema. L-Dex scores were highly correlated with the difference in arm volume measured by perometry. Thus, L-Dex provides a measurement index that is highly correlated ($r = 0.926$) with quantitative measures of the volume increase in limb size seen in lymphedema.

The L-Dex parameter uses the measured limb ratio to give an indication of the degree of difference of the measured limb ratio from a normal healthy population limb ratio. L-Dex values that lie outside the normal range (-10 to 10) or that have changed $+10$ L-Dex units from baseline may indicate early signs of lymphedema. Due to the increase in the natural spread of the impedance ratio in a normal population, the sensitivity of the method is expected to decrease as the measurement frequency increases and there becomes less clear separation between a control subject and a lymphedema subject.

The spread of the L-Dex parameter for the control data, along with the spread of the L-Dex parameter for the sample lymphedema populations, is shown visually through a box and whisker plot in Figure 2 for a collection of frequencies for arms and legs. The L-Dex score is calculated at each frequency for each subject as an indicator of lymphedema based on gender and limb dominance. This allows all subjects to be plotted on a single graph, despite these differences. The box and whisker plot displays the sample minimum, the lower quartile, the median, the upper quartile, and the sample maximum of the population.

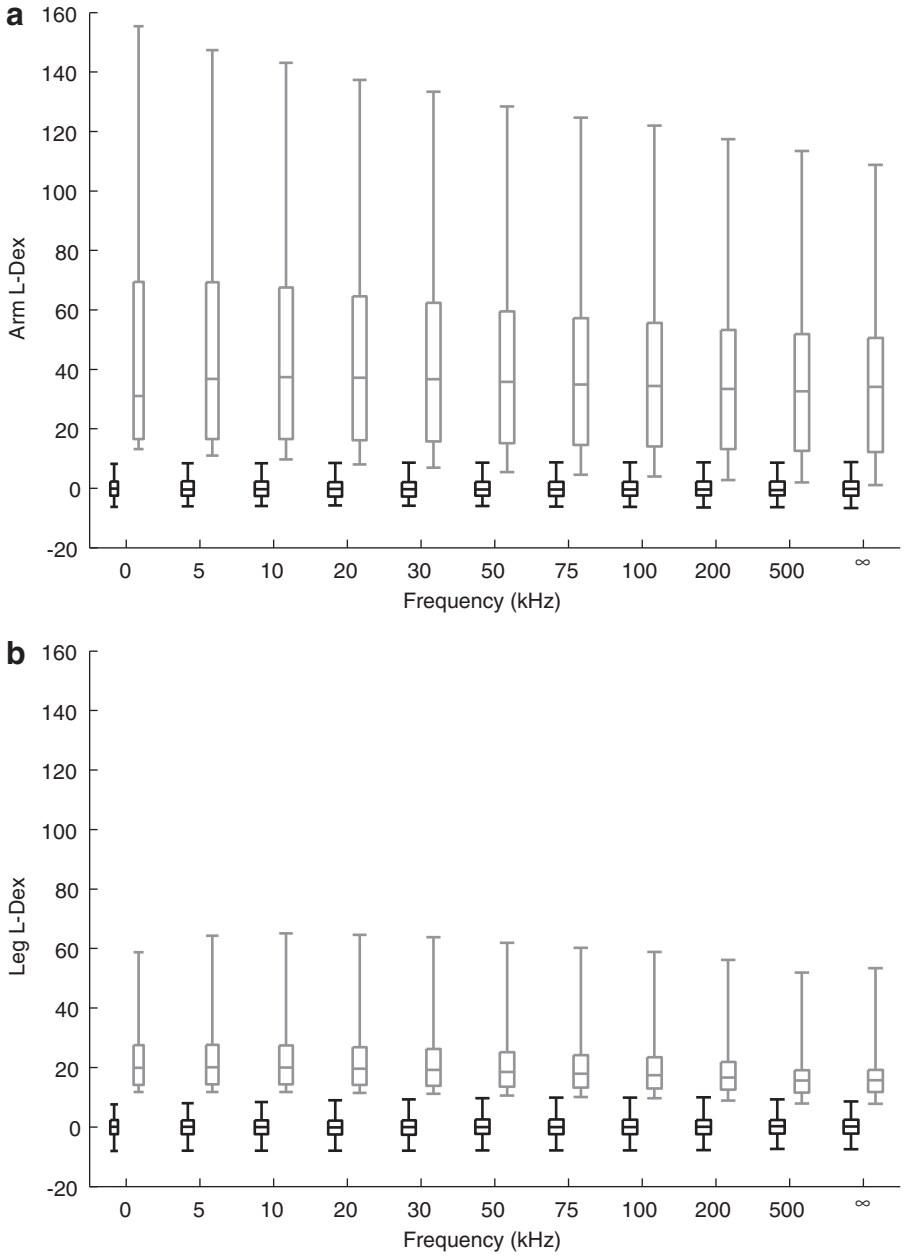

FIG. 2. Box and whisker plots demonstrating the spread of L-Dex at a number of increasing frequencies for **(a)** control (L) and clinically evident unilateral arm lymphedema subjects (R) and **(b)** control (L) and clinically evident unilateral leg lymphedema subjects (R)

Figure 2 clearly shows that there is separation between the control and test group at R0 for both arms and legs. This indicates good differentiation between lymphedema subjects and unaffected subjects for an L-Dex calculated from R0. At about 20–30 kHz, an overlap between the upper quartile of the normal population and the lower quartile of the affected arm population becomes evident. This overlap increases as the measurement frequency increases. In the case of the arms, the overlap in impedance ratio between the population groups is larger than for the legs. In both the arm and leg cases, the overlap between control and test groups becomes large enough to reduce the sensitivity of assessment above 30 kHz.

This indicates that impedance measurements above 30k kHz are not sufficiently sensitive to extracellular fluid changes to be used effectively to assess early stages of lymphedema accurately. This is because the electric current will pass through a combination of both extra- and intracellular fluids at these frequencies. The assessment of lymphedema using BIS has been reported[14] and has shown that the extracellular fluid volumes of the arm calculated from R0 are more sensitive in determining the presence of lymphedema than total body fluids of the arm calculated from the characteristic frequency, Zc (usually around 50–80 kHz). The study showed that the sample populations of the total fluid arm ratio for the control group and the lymphedema group overlapped.

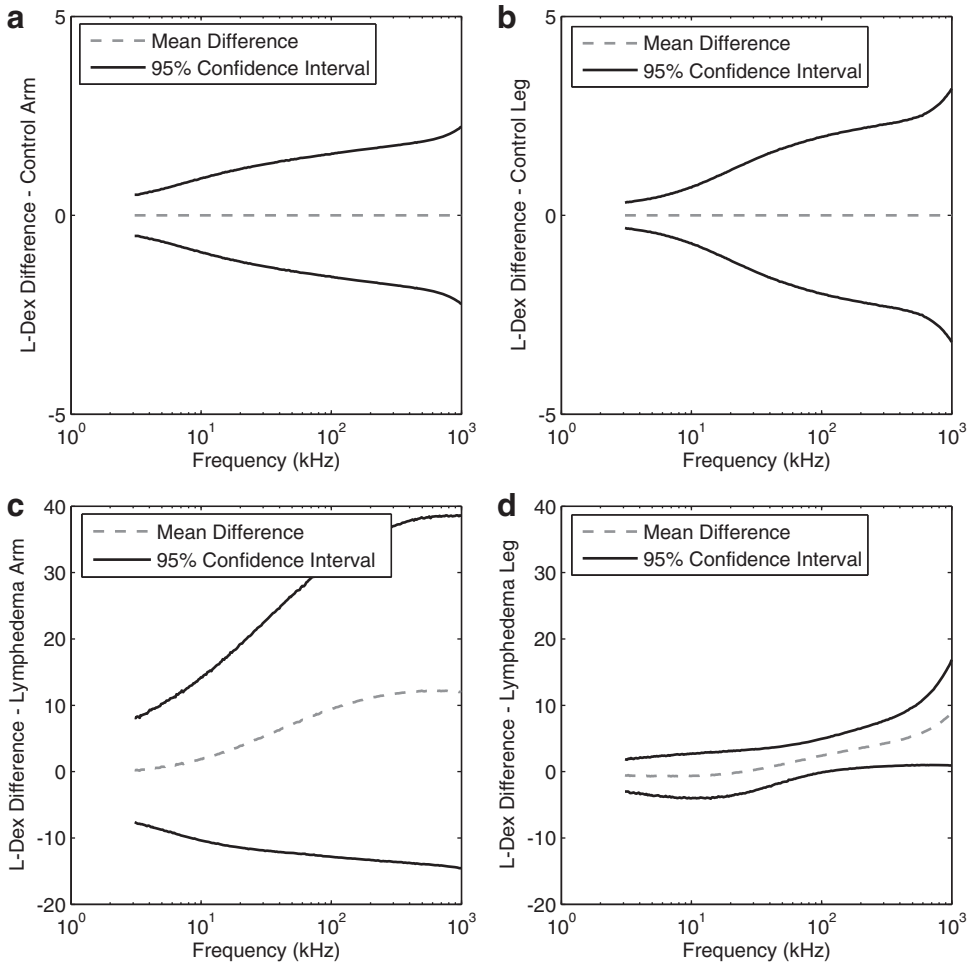

FIG. 3. Summary of Bland and Altman mean difference from L-Dex calculated from R_0 (- -) and 95% confidence intervals (-) as a function of frequency for **(a)** control subject arms, **(b)** control subject legs, **(c)** test lymphedema subject arms, and **(d)** test lymphedema subject legs.

However, clear discrimination between the two subject groups is clearly shown for extracellular fluid ratios of the arms. Therefore, bioimpedance devices that measure the total fluid of a limb will not be sensitive to changes in lymphedema.

High concordance ($r_c > 0.973$ for frequencies below 200 kHz) has been reported[6] between impedance ratios calculated at a specific frequency from those calculated at R_0 for both arms and leg. However, widening of the 95% confidence interval and an increased bias are also reported for six increasing frequencies, based on Bland and Altman limits of agreement. This previously reported method of analysis was used with the data collected in the current study. Agreement between the L-Dex calculated at a specific frequency and the L-Dex calculated at R_0 was determined using a Bland and Altman analysis for each of 256 frequencies from 3 kHz to 1000 kHz. A summary of the mean difference and the 95% confidence interval as a function of frequency is shown in Figure 3, separating the arms and legs of the control population, the arm lymphedema population, and the leg lymphedema population.

The mean difference between the L-Dex calculated from R_0 and the L-Dex calculated for each frequency for both the arms and the legs of the control population remains constant at close to zero for the entire frequency range. The mean difference for both the arms and legs of the lymphedema population is shown to increase as frequency increases (0.1 to 12 for arms and −0.5 to 9 for legs over the frequency range 3 kHz–1000 kHz). This is because of the presence of fibrotic tissue expected to be present in an established lymphedema population such as the one used in this study. At low frequencies, the measurement of impedance comprises primarily extracellular fluid, while impedance measurements made at high frequencies include interactions from the cells and thus comprises both tissue and extracellular fluid components. Thus the increasing difference between L-Dex at higher frequencies and the L-Dex calculated from R_0 is due to the presence of fibrosis which is not present in a healthy population. The bias of the mean difference at high frequencies highlights the importance of using low frequency impedance for the assessment of lymphedema in order to detect the onset of the disease early and avoid irreversible damage.

Each population also displays a large widening of the 95% confidence intervals. This is larger in the lymphedema populations. This further demonstrates that the sensitivity of the method decreases as the current frequency increases.

Conclusions

R_0 has been shown previously as the best measurement frequency to assess lymphedema of the arms, as it is more accurate in measuring the extracellular water content of a limb.[3] The present study has shown that the standard deviation of contralateral limb impedance ratios in a normal population increases as the measurement frequency increases. As such, cut off criteria used to identify the onset of lymphedema also increases and sensitivity is reduced.

The spread of the control L-Dex and the L-Dex calculated for both arm and leg test subjects, has also been shown to increase with frequency. The frequency at which an overlap occurs between the lower quartile of the test group L-Dex and the upper quartile of the control group L-Dex begins at about 30 kHz. This results in a decreased sensitivity for the detection of lymphedema above this frequency.

A comparison between the L-Dex calculated at 0 kHz and the L-Dex calculated at any non-zero single frequency shows that the mean difference between the methods remains constant over the frequency range in a healthy population and increases in the test lymphedema populations. This demonstrates the importance of using low frequency impedance measurements to provide an accurate assessment of the presence of lymphoedema and provides further supporting evidence that the current frequency used in bioimpedance should be below 30 kHz to accurately assess unilateral lymphedema. The 95% confidence interval was also shown to increase with frequency for each population group. This again shows an improved sensitivity in assessing lymphedema at low measurement frequencies.

The many single frequency body composition devices available on the market traditionally measure the impedance at 50 kHz. This study shows that the application of these devices to the assessment of lymphoedema is less sensitive to extracellular fluid changes. It has been demonstrated that changes in the extracellular fluid in unilateral lymphedema patients (both arm and leg) can only be monitored accurately and reliably using bioimpedance devices with BIS capabilities or applied current frequencies below 30 kHz.

Acknowledgments

The research studies have been funded by in kind support from ImpediMed Ltd, Brisbane, Australia, with clinical measurements of test subjects obtained independently by Dr. R Box.

Author Disclosure Statement

At the time of data collection, analysis, and reporting, R. Gaw was also employed at ImpediMed Limited on a part-time basis. To ensure no conflict of interest existed, clinical measurements of test subjects were obtained independently by Dr. R. Box, measurements of control subjects were obtained by R. Gaw *and* Prof. B. Cornish, and data analysis was performed by R. Gaw *and* Prof. B. Cornish. No other competing financial interests exist.

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1. Stanley G. Rockson . 2011. Maturational Aspects of Postnatal LymphangiogenesisMaturational Aspects of Postnatal Lymphangiogenesis. *Lymphatic Research and Biology* **9**:2, 75-75. [Citation] [Full Text] [PDF] [PDF Plus]